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[Optimized codon usage enhances the expression and immunogenicity of DNA vaccine encoding the HPV 6b E7 gene]

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Zhao W, Yu X, Zhou Y, Bian J, Jia J, Luan Y, Qi M, Sun X, Wang H.

Department of Microbiology, School of Medicine, Shandong University,
Jinan 250012, China.

OBJECTIVE: To analyze the influence of optimal codon usage on the expression levels and immunogenicity of DNA vaccines, encoding the human papillomavirus type 6b (HPV 6b) E7 gene. **METHODS:** The full length E7 gene of HPV 6b was modified to substitute human preferred codon for rarely used codon, and three mutations were introduced into the pRB binding site of HPV 6b E7 to eliminate its transformation potential. The codon optimized and mutated E7 gene (hu-mE7) were cloned into the Kpn I and EcoR I site of the pcDNA3 mammalian expression vector, the in vitro expression of the hu-mE7 gene and the immunogenicity of hu-mE7 DNA vaccine were compared with the wt-E7 gene. **RESULTS:** The in vitro expression of pcDNA3-hu-mE7 was much higher than the classical wt-E7 plasmid in monkey COS-1 cell line. Mice immunized intramuscularly with the pcDNA3-hu-mE7 showed that the codon modified E7 gene induced a stronger IFN-gamma ratios than the wt-E7 gene. **CONCLUSIONS:** These results suggest that the optimized codon usage contributes to the enhancement of gene expression and immunogenicity of HPV 6b E7 gene.

PMID: 12665891 [PubMed - indexed for MEDLINE]

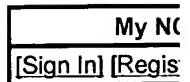
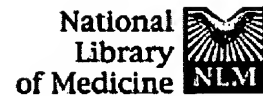
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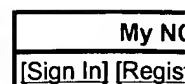
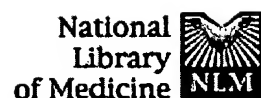
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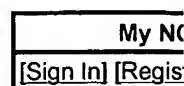
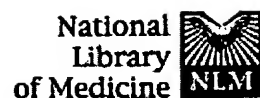
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[Immunogenicity study of HPV 6b virus-like particles]

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Liu Y, Liu X, Frazer IH.

Department of Dermatology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China.

OBJECTIVE: To confirm human papillomavirus (HPV) 6b virus-like particles (VLP) have strong immunogenicity and the protective antibody induced by HPV 6b VLP have cross-reactive immunity against HPV11 VLP and bovine papillomavirus (BPV) 1 VLP. **METHOD:** The late gene L1 for HPV6b, HPV 11 and L1/L2 for BPV 1 were molecularly cloned into recombinant baculovirus, respectively. The recombinant viruses were expressed in insect cells (Sf-9 cells). The expressed L1 proteins self-assembled into virus-like particles (VLP) for HPV6b, HPV 11 and BPV 1. VLP were purified from insect cell nuclei by CsCl centrifugation. The Balb/c mice were immunized on days 0 and 21 with 50 microgram HPV6b VLP intramuscularly. Sera were collected after a further 7 days and 3 months. The titers of IgG against HPV 6b VLP, HPV 11 VLP and BPV 1 VLP were detected. Hemagglutination inhibition assay was conducted to detected that whether antisera produced by HPV 6b VLP immunization could inhibit HPV11 VLP and BPV 1 VLP agglutinate mouse red blood cells. **RESULT:** After 7 days of two immunizations, the titers of IgG against HPV6b VLP, HPV11 VLP and BPV1 VLP were 1:6 400, 1:1 600 and 1:1 600 by ELISA, respectively. Three months later, the titers of IgG against HPV6b VLP, HPV11VLP and BPV1 VLP were 1:800, 1:400 and 1:100, respectively. Hemagglutination inhibition assay results showed that the antisera produced by HPV6b VLP inhibit HPV6b VLP and HPV11 VLP to mouse red blood cells binding. **CONCLUSION:** HPV 6b VLP have potent immunogenicity. Antisera produced by HPV6b VLP could inhibit the binding of HPV6b VLP and HPV11 VLP and cells. Both HPV6b and HPV11 share neutralizing epitopes which are cross-reactive and HPV6b VLP may be used in prophylactic and therapeutic vaccine for HPV6b and/or HPV 11 infections.

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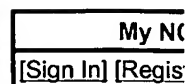
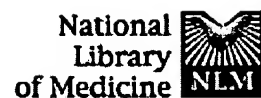
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1: J Virol. 1992 Apr;66(4):2008-19.

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Fred Hutchinson Cancer Research Center, Seattle, Washington 98104-2092.

Human serum samples derived from a case-control study of patients with cervical carcinoma (n = 174) or condyloma acuminatum (n = 25) were tested for the presence of immunoglobulin G antibodies to human papillomavirus type 6 (HPV6) L2 and HPV11 L2 recombinant proteins in a Western immunoblot assay. Thirty-six samples (18%) were positive for HPV6 L2 antibodies alone, 25 (13%) were positive for HPV11 L2 antibodies alone, and 34 (17%) were positive for both HPV6 L2 and HPV11 L2 antibodies. Thirty samples that were positive for both antibodies were tested for the presence of HPV6-HPV11 L2 cross-reactive antibodies. Fifteen (50%) serum samples contained HPV6-HPV11 L2 cross-reactive antibodies, and 15 (50%) contained independent, type-specific HPV6 L2 and HPV11 L2 antibodies. Altogether, 82% of the HPV6 L2 and HPV11 L2 antibody reactivities were type specific and 18% were HPV6-HPV11 cross-reactive. There was no significant difference in the prevalence of antibody reactivities between samples from patients with cervical carcinoma and those with condyloma acuminatum. Deletion mapping identified five HPV6 L2 regions that reacted with HPV6 type-specific antibodies: 6U1 (amino acids [aa] 152 to 173), 6U2 (aa 175 to 191), 6U3 (aa 187 to 199), 6U4 (aa 201 to 217), and 6U5 (aa 351 to 367). Five HPV11 L2 regions that reacted with HPV11 type-specific antibodies were identified: 11U1 (aa 49 to 84), 11U2 (aa 147 to 162), 11U3 (aa 179 to 188), 11U4 (aa 180 to 200), and 11U5 (aa 355 to 367). Two HPV6-HPV11 cross-reactive regions were identified: 6CR1 (HPV6 L2 aa 106 to 128)/11CR1 (HPV11 L2 aa 103 to 127) and 6CR2 (HPV6 L2 aa 187 to 199)/11CR2 (HPV11 L2 aa 180 to 200).

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DOCUMENT-IDENTIFIER: US 6887478 B2

TITLE: Formalin-treated human papillomavirus L1 protein vaccine

DATE-ISSUED: May 3, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
<u>Schlegel; C. Richard</u>	Rockville	MD		
Jenson; A. Bennett	Rockville	MD		
Ghim; Shin-je	Washington	DC		

US-CL-CURRENT: 424/204.1; 530/300, 536/23.72

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw. D.
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☐ 2. Document ID: US 6485728 B2

L10: Entry 2 of 3

File: USPT

Nov 26, 2002

US-PAT-NO: 6485728

DOCUMENT-IDENTIFIER: US 6485728 B2

TITLE: Formalin-Inactivated human papillomavirus L1 protein vaccine

DATE-ISSUED: November 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
<u>Schlegel; C. Richard</u>	Rockville	MD		
Jenson; A. Bennett	Rockville	MD		
Ghim; Shin-je	Washington	DC		

US-CL-CURRENT: 424/204.1; 424/184.1, 424/186.1, 424/199.1, 536/23.72

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw. D.
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☐ 3. Document ID: US 5874089 A

L10: Entry 3 of 3

File: USPT

Feb 23, 1999

US-PAT-NO: 5874089

DOCUMENT-IDENTIFIER: US 5874089 A

**** See image for Certificate of Correction ****

TITLE: Protecting against canine oral papillomavirus (copy)

DATE-ISSUED: February 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
<u>Schlegel; C. Richard</u>	Rockville	MD		
Jenson; A. Bennett	Rockville	MD		
Ghim; Shin-je	Washington	DC		

US-CL-CURRENT: 424/204.1; 424/184.1, 424/186.1, 424/192.1, 424/199.1, 435/235.1,
435/320.1, 435/5, 435/69.1, 435/69.3, 536/23.72

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw D
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DATE: Monday, August 29, 2005

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<input type="checkbox"/>	L11	Ertl.in. and papilloma	5
		<i>DB=USPT; PLUR=YES; OP=ADJ</i>	
<input type="checkbox"/>	L10	Schlegel C Richard.in.	3
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<input type="checkbox"/>	L4	HPV-6 and HPV-11.clm.	14
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<input type="checkbox"/>	L2	HPV6 and HPV11.clm.	7
<input type="checkbox"/>	L1	HPV6 and HPV11	63

END OF SEARCH HISTORY